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Molecular Screening of Blood Donors for *Babesia* in Tyrol, Austria

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Keywords

Babesia · Polymerase chain reaction · Screening · Blood donor · Blood transfusion · Austria

Abstract

Introduction: *Babesia* is a tick-borne intraerythrocytic parasite that is globally ubiquitous, yet understudied. Several species of *Babesia* have been shown to be transfusion-transmissible. *Babesia* has been reported in blood donors, animals, and ticks in the Tyrol (Western Austria), and regional cases of human babesiosis have been described. We sought to characterize the risk of *Babesia* to the local blood supply. ***Methods:*** Prospective molecular testing was performed on blood donors who presented to regional, mobile blood collection drives in the Tyrol, Austria (27 May to October 4, 2021). Testing was conducted using the cobas® Babesia assay (Roche Molecular Systems, Inc.), a commercial PCR assay approved for blood donor screening that is capable of detecting the 4 primary species causing human babesiosis (i.e., *B. microti*, *B. divergens*, *B. duncani*, and *B. venatorum*). A confirmatory algorithm to manage initial PCR-reactive samples was developed, as were procedures for donor and product management. ***Results:*** A total of 7,972 donors were enrolled and screened; 4,311 (54.1%) were male, with a median age of 47 years (IQR = 34–55). No positive cases of *Babesia* were detected, corresponding with an overall prevalence of 0.00% (95% CI: 0.00%, 0.05%). ***Discussion:*** The findings suggest that the prevalence of *Babesia* is low in Austrian blood donors residing in the Tyrol, even during months of peak tick exposure. Although one cannot conclude the absence of *Babesia* in this population given the limited sample size, the findings suggest that the regional risk of transfusion-transmitted babesiosis is low.

Introduction

Babesiosis is the symptomatic infection with any of the members of the *Babesia* genus, a group of tick-borne, intraerythrocytic protozoan parasites [1]. Most cases of human babesiosis are attributed to *Babesia microti*, a species that is known to be transfusion-transmissible [2]. Asymptomatic *Babesia* infection is well described and can persist for months to years in some individuals, thus posing risk of not being detected at the time of blood donation, particularly in the absence of laboratory-based donor testing [3]. Although infection may be mild or subclinical in healthy adults, certain patient groups such as neonates, individuals aged >50 years, the asplenic, and the immunocompromised (HIV, cancer, and immuno-

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suppressant therapy) are at risk of severe or even fatal babesiosis [1]. These same populations are over-represented among transfusion recipients, which likely accounts for the high all-cause mortality (~19%) associated with transfusion-transmitted babesiosis [4].

A 2014 seroprevalence study of Tyrolean blood donors (n = 988) [i.e., in Western Austria] found that 2.1% of donors demonstrated IgG antibodies against the *B. divergens* complex and 0.6% were seropositive against *B. microti* [5]. Regional surveillance and case reporting indicate that *Babesia* is present in humans, ticks, and animal populations in Europe [6–11], although rare, transfusion-transmitted babesiosis has also been described in Europe [8]. We conducted a *Babesia* molecular surveillance study of blood donors in Tyrol, Austria, to characterize the risk of *Babesia* to the local blood supply.

Materials and Methods

Overview of Population and Setting

The study was conducted in the Tyrol region of Western Austria, where both *B. divergens* and *B. microti* have been reported [5].

Sampling and Eligibility

Consecutive sampling was conducted of all community whole blood (WB) donors who presented during the study period (27 May to October 4, 2021) and who consented to participate. Direct-, autologous-, and apheresis platelet/plasma donors were excluded as were individuals with a reported tick bite in the 4 weeks prior to donation. Donor demographics (age, sex, area of residence) were captured at the blood center in Innsbruck, but no personal identifiers were shared with the research personnel, thus protecting donor confidentiality.

Laboratory Processing and Babesia PCR Testing

At the time of the donor visit, trained nurses or technicians collected approximately 1 mL of WB into a Roche WB collection tube specifically for *Babesia* testing. The proprietary collection tube draws up to 1.1 mL of WB into 7.7 mL of a chaotropic reagent that lyses red blood cells and preserves nucleic acid [12].

Babesia PCR

The donors' samples, comprising 850 µL of lysed WB, were tested individually (i.e., single donor specimens) using the cobas® Babesia test (Roche Molecular Systems Inc., Pleasanton, CA). The cobas® Babesia test is a qualitative real-time reverse transcriptase-polymerase chain reaction test capable of detecting four species of *Babesia*: *B. microti*, *B, duncani*, *B. divergens*, and *B. venatorum* [12]. The cobas® Babesia assay is FDA-licensed and CE-marked for blood donor screening. The assay's performance characteristics have previously been described: the reported analytic sensitivity for *B. microti* and *B. divergens* is 6.1 (95% confidence interval [CI]: 5.0, 7.9) and 26.1 (95% CI: 22.3, 31.8), infected red blood cells/mL, respectively. The clinical specificity is 99.999% [12]. The test was run on the cobas® 6800/8800 Systems at the Bavarian Red Cross. The testing was performed in real time, whereby a negative test was required before releasing the associated unit of blood (i.e., from quarantine). Repeat and confirmatory testing was planned using a combination of the cobas® Babesia test, indirect fluorescent antibody testing and sequencing.

Table 1. Characteristics of the study population

	No. of donors (%)[a] (n = 7,972)	No. of donations (%) (n = 8,028)
Study month		
May	1 (0.0)	1 (0.0)
June	686 (8.6)	696 (8.7)
July	2,868 (36.0)	2,912 (36.3)
August	2,128 (26.7)	2,130 (26.5)
September	2,124 (26.6)	2,124 (26.5)
October	165 (2.1)	165 (2.1)
Country		
Austria	7,962 (99.9)	8,018 (99.9)
Germany	8 (0.1)	8 (0.1)
Italy	2 (0.0)	2 (0.0)
Median age (IQR), years	47 (34–55)	47 (34–55)
Age group, years		
18–24	804 (10.1)	812 (10.1)
25–34	1,286 (16.1)	1,297 (16.2)
35–44	1,446 (18.1)	1,456 (18.1)
45–54	2,197 (27.6)	2,214 (27.6)
55–64	1,880 (23.6)	1,887 (23.5)
≥65	359 (4.5)	362 (4.5)
Sex		
Female	3,661 (45.9)	3,688 (45.9)
Male	4,311 (54.1)	4,340 (54.1)
Blood group		
A	3,215 (40.3)	3,240 (40.4)
B	770 (9.7)	774 (9.6)
AB	293 (3.7)	298 (3.7)
O	3,694 (46.3)	3,716 (46.3)

[a] For donor-level data, characteristics were ascertained from the most recent blood donation for donors that presented more than one time during the study period.

Statistical Analysis

Descriptive statistics were used to characterize the study sample of blood donations and blood donors. For donor-level data, characteristics were ascertained from the most recent blood donation for donors that presented more than one time during the study period. The prevalence of *Babesia* and corresponding Clopper-Pearson 95% CIs were estimated at the donor level. Data analysis was conducted in Stata/MP, version 15.1 (StataCorp, College Station, TX).

Human Subjects

The study was approved by the Ethics Committee of the Medical University of Innsbruck (1090/2021) prior to study initiation.

Results

The study sample included 8,028 blood donations collected from 7,972 unique blood donors (56 donors contributed two blood donations) at 71 collection sites (May to October 2021). Of the 11,711 available donors, 7,972 took part in the study, yielding a participation rate of 68.07%. The characteristics of the sampled donor popula-

tion are provided in Table 1. Of the 8,028 blood donations examined, there was no reactive sample for *Babesia*. At the blood donor level (n = 7,972), the overall prevalence of *Babesia* was 0.00% (95% CI: 0.00%, 0.05%).

Discussion

Our study, in which over 8,000 blood donations across the Tyrol were tested for *Babesia* by PCR, found none to be positive for *Babesia*. The findings suggest that the current risk to the local blood supply, if any, is low.

To date, surveillance for *Babesia* outside of the USA has been very limited [2]. The few studies in blood donors that have been conducted outside of the USA have found the prevalence of *Babesia* to be low or even absent in the individual populations that were surveyed [13, 14]. Molecular surveillance has been the exception; one study in Canada found 1 of 50,752 donation samples to be positive [15].

Despite our study's negative finding, tick-borne diseases – including babesiosis – appear to be on the rise [16–18]. Contributing factors include an increase in populations of ticks and associated interaction with their hosts [19–22]. Climate change and global warming have complex, yet unpredictable effects on vectors and vector-borne diseases [23, 24]. There may be changes in how local habitats support tick populations [25]. The birth rates and development of ticks are notably impacted favorably by an increase in temperature [26]. Shorter tick development times due to increased habitat temperatures and increased feeding opportunities for ticks may increase the possibility of human and non-human host infections by *Babesia* [27, 28]. Birds can also carry ticks – including those that are infected – thus introducing tick-borne diseases to areas that have not – historically – been considered to be endemic [29].

This study had limitations. It is still possible that *Babesia* is present but was missed due to the small sample size and/or short observation period. To place the findings in context, in a study of 89,153 blood donations originating in four states in the USA where *B. microti* is highly endemic, 67 donors (i.e., 1 in 1,331) were PCR positive for *Babesia* [30]. Second, serology was not performed; rather, it was planned in the event that PCR-positive cases were detected. While serology offers a better estimate of exposure, molecular methods correlate better with parasitemia and the associated risk of transfusion transmission [30]. Third, testing of Tyrolean *Babesia* samples has not been performed, previously using the cobas® Babesia assay. However, the test has been validated to detect *B. divergens* and *B. microti*, for which evidence of population exposure was found in the previous serosurvey [5]. Molecular testing has advantages over serological testing, thus supporting the rationale for the adoption of molecular screening of blood donors in high-risk areas of the USA. The discordance between our results with those of the previous serosurvey [5] is unsurprising [2]. A high proportion (almost 90%) of infected individuals resolve infection within 1 year of an index positive test (i.e., as reflected by negative molecular testing). By contrast, less than 10% of individuals will serorevert within the same timeframe [30]. There is also the possibility of prior overestimation of the prevalence [5] given challenges surrounding the specificity of antibody-based assays for *Babesia*. Further, donors who reported a tick bite during the 4 weeks prior to donation would have been excluded, diminishing the probability for finding a NAT-positive donation in our study.

In conclusion, no cases of *Babesia* infection – as determined by molecular testing – were found in a limited surveillance study of blood donors in Tyrol, Austria. The findings support vigilance rather than laboratory-based donor screening at this time.

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Statement of Ethics

The study was reviewed and approved by the Ethics Committee of the Medical University of Innsbruck (1090/2021) prior to study initiation. Written informed consent was obtained from all subjects who were involved in the study.

Conflict of Interest Statement

Evan M. Bloch reports personal fees and non-financial support from Terumo BCT, Abbott Laboratories, Tegus, and UpToDate, outside of the submitted work. Steven J. Drews is a paid consultant to Roche and has received research support from Abbott, outside of the submitted work. Harald Schennach reports personal fees from Cerus Corp outside of the submitted work. Anita Siller, Laura Tonnetti, Bryan Spencer, Doris Hedges, Tessa Mergenthal, Marijke Weber-Schehl, Manfred Astl, Eshan Patel, and Manfred Gaber have no conflict of interest to declare.

Evan M. Bloch is a member of the US Food and Drug Administration (FDA) Blood Products Advisory Committee. Any views or opinions that are expressed in this manuscript are those of the author's, based on his own scientific expertise and professional judgment; they do not necessarily represent the views of either the Blood Products Advisory Committee or the formal position of FDA, and do not bind or otherwise obligate or commit either Advisory Committee or the Agency to the views expressed.

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Author Contributions

The following roles were assumed by the individual co-authors: study conception and design (Evan M. Bloch, Anita Siller, Laura Tonnetti, Steven J. Drews, Bryan R. Spencer, and Harald Schennach), subject enrollment and testing (Doris Hedges, Tessa Mergenthal, Marijke Weber-Schehl, and Manfred Gaber), data analysis and interpretation (Eshan U. Patel [lead], Evan M. Bloch, Anita Siller, Laura Tonnetti, Laura Tonnetti, Steven J. Drews, Bryan R. Spencer, Manfred Astl, and Harald Schennach), writing first draft of manuscript (Evan M. Bloch, Anita Siller, and Harald Schennach), and review and editing (all co-authors).

Data Availability Statement

A deidentified dataset will be made available on request. Participant-level data on the blood donor cohort cannot be shared due to regulatory restrictions.

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